

Up and away: ontogenic transference as a pathway for aerial dispersal of microplastics

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1 **Up and away: ontogenic transference as a pathway for aerial dispersal of**
2 **microplastics**

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Abstract

Microplastics (MPs) are ubiquitous pollutants found in marine, freshwater and terrestrial ecosystems. With so many MPs in aquatic systems it is inevitable that they will be ingested by aquatic organisms, and be transferred up through the food chain. However, to date, no study has considered whether MPs can be transmitted by means of ontogenic transference i.e. between life stages that utilise different habitats. Here, we determine whether fluorescent polystyrene beads could transfer between *Culex* mosquito life stages and, particularly, could move into the flying adult stage. We show for the first time that MPs can be transferred ontogenically from a feeding (larva) into a non-feeding (pupa) life stage and subsequently into the adult terrestrial life stage. However, transference is dependent on particle size, with smaller 2µm MPs transferring readily into pupae and adult stages, whilst 15µm MPs transferred at a significantly reduced rate. Microplastics appear to accumulate in the Malpighian tubule renal excretion system. The transfer of MPs to the adults represents a potential aerial pathway to contamination of new environments. Thus, any organism that feeds on terrestrial life phases of freshwater insects could be impacted by MPs found in aquatic ecosystems.

Keywords

Food chain: ontology; life stage; Malpighian tubules, microplastics; *Culex pipiens*

53

54 **Introduction**

55 Microplastics (MPs) are ubiquitous pollutants found in marine, freshwater and terrestrial
56 ecosystems [1–3]. There is little doubt that plastic and MP pollution is a major
57 environmental concern globally. Despite this, there is relatively little research into the impact
58 of MPs on freshwater ecosystems, with most research concentrating on marine systems and
59 organisms [2]. MPs have been defined as plastic particles smaller than 5mm in size [4,5].
60 However, this simple description covers a wide range of types, including, among others,
61 polypropylene, polyethylene and polystyrene MPs entering the environment in different
62 shapes and sizes, including fibres, pellets and cosmetic beads [6,7]. MPs are categorised
63 based on their origin as primary or secondary types, depending on whether they were
64 released into the environment as MPs (primary) or have degraded to that size in the
65 environment (secondary) [8,9]. Microplastics pass through terrestrial environments in
66 household wastewater [2,10]. Rivers can subsequently deliver MPs into the sea and lakes,
67 where they can be found in high concentrations [11–13].

68

69 Microplastics are ingested by aquatic organisms, and can be transferred through the food
70 chain in both freshwater and marine environments [14–18]. However, to date no study has
71 considered whether MPs can be transmitted by means of ontogenic transference i.e. between
72 life stages that utilise different habitats. Freshwater environments are inhabited by insects that
73 spend their juvenile stages in water but their adult stages in the terrestrial environment. Such
74 insects include mayflies, dragonflies, midges and mosquitoes, most of which are eaten by
75 terrestrial vertebrates. This raises the potential for MPs to enter terrestrial ecosystems from
76 freshwater habitats aerially *via* transference to adult invertebrate life stages. Here, we thus
77 determine whether 2 and 15µm fluorescent polystyrene beads could transfer between insect

life stages and, particularly, could move into the flying adult stage. Fluorescent beads were selected to enable MPs to be easily detected in the non-feeding stages and also to allow an investigation of location within the body during metamorphosis. The *Culex pipiens* mosquito complex was selected as a model for this study given their worldwide distribution and broad habitat preference [19]. Mosquitoes develop through four feeding larval instars and a non-feeding pupal stage, and finally emerge into a flying adult.

Materials and methods

For additional details of all methods and analyses, see the electronic supplementary material. Two types of MPs were used: a 2µm fluorescent yellow-green carboxylate-modified polystyrene (density 1.050g/cm³, excitation 470nm; emission 505nm, Sigma-Aldrich, UK) and a 15.45±1.1µm fluorescent dragon green polystyrene (density 1.06 g/cm³ (5x10⁶ particles/ml, excitation 480nm; emission 520nm, Bangs Laboratories Inc., USA). Four treatments were used; a control with no microplastics, a treatment of 8x10⁵ 2µm particles/ml, a treatment of 8x10² 15µm particles/ml, and a 1:1 mixture of both treatments. Each replicate (five per treatment) contained ten 3rd instar *C. pipiens* larvae in a 50ml glass beaker filled with 50ml of tap water. The control and all treatments contained 100mg of pelleted guinea pig food. Treatments were assigned randomly to a position on the laboratory bench to reduce experimental error.

One random individual was removed from each beaker when every mosquito had moulted into the 4th instar, and again when they pupated or emerged as adults. All samples were then placed in separate 1.5ml Eppendorf tubes and stored at -20 °C prior to examination. Microplastics were extracted from mosquitoes by homogenization and filtration. The filter membrane was examined using an epi-fluorescent microscope (Zeiss Axioskop) under a 20x lens to count the number of fluorescent MPs. Adults were further dissected under a binocular

103 stereo microscope (0.7X-4.5X) to extract the gut and quantify the numbers of MPs under the
104 epi-fluorescent microscope [20].

105 All data were analyzed using the statistical software R v3.4.2 [21]. Microplastic counts were
106 analysed using generalized linear models (GLMs) assuming a quasi-Poisson distribution.

107 Uptake of microplastics was examined with respect to 'particle size', 'treatment' and 'life
108 stage'. We performed model simplification via stepwise removal of non-significant effects.

109 Tukey tests were used post hoc for multiple comparisons.

111 Results

112 No MPs were found in control groups of any mosquito life stage. Densities of MPs were
113 significantly different between life stages ($F_{2, 56}=160.42$, $P<0.001$), with MP numbers
114 significantly falling as mosquitoes moved between successive ontogenic levels (all $P<0.001$)
115 (Figure 1, Table S1, S2). Microplastic transference to adults was confirmed by fluorescent
116 microscopy where the beads were detected in the adult abdomen, specifically inside the
117 Malpighian tubules (Figure 2).

118 Significantly more 2 μ m particles were found in mosquito life stages than 15 μ m particles
119 overall ($F_{1, 58}=303.98$, $P<0.001$). Microplastics uptake was also significantly greater overall
120 in mixed exposure treatments ($F_{1, 55}=6.00$, $P=0.02$). Although 2 μ m particles were transferred
121 to adults in all instances, we found no transference of 15 μ m particles following single
122 treatment exposures. However, in the mixed MPs treatment, transference to adults of both
123 2 μ m and 15 μ m particles was evidenced (Figure 1).

125 Discussion

126 Here, we show for the first time that MPs can be transferred ontogenically from a feeding
127 (larval) into a non-feeding (pupal) life stage and subsequently into the flying (adult) life

stage. Transference through to adults was found in both MP sizes, although the larger 15µm MPs were not ingested as readily as the 2µm MPs. Dissection of mosquito adults showed that 2µm MPs accumulated in the renal excretion system of Malpighian tubules which, unlike the gut, pass from larvae to adult stages without visible reorganization [22]. This has been demonstrated previously to provide a physical transport system between stages during metamorphosis for *Pseudomonas* bacteria and seems to be important for ontogenic transmission from larvae to adults [23].

Few 15µm MPs were transferred into adults suggesting that MP size is an important factor in ontogenic transfer which could be related to the transfer and accumulation of MPs in the Malpighian tubes. Although the translocation mechanism of MPs to the Malpighian tubules is unclear in mosquitoes, analysis of fish, fiddler crab and marine mussels has demonstrated that MPs can be translocated from gastrointestinal tracts into other tissues in a wide range of phyla [24, 25,26]. Malpighian tubules have an entry point to the gut between the mid- and hindgut of mosquitoes, but the flow of fluid is from the Malpighian tubules to the hindgut [27]. Diptera are known to produce structures called concretions in the Malpighian tubules which have been shown to sequester heavy metals [28]. However, it is unlikely that this pathway would operate with a solid MP.

Our results have important implications since any aquatic life stage that is able to consume MPs and transfer them to their terrestrial life stage is a potential vector of MPs onto novel aerial and terrestrial habitats. Ingestion of MP-contaminated organisms by terrestrial organisms is not new [29]. Indeed, the widespread distribution of MPs in marine environments has meant that animals such as fish and shellfish sold for human consumption are contaminated with a range of plastics with a consequent transference of MPs between trophic levels [24]. Unlike MP fibres, which are common in the air and atmosphere, there has been no evidence for MPs being transported into the air [24]. We have demonstrated here

that species with aquatic and terrestrial life stages can harbour MPs through their life history. Adults are predated on emergence by many animals including dipteran flies Empididae and Dolichopodidae, whilst resting predominantly by spiders and in flight they are the prey of dragonflies, damselflies, birds (such as swallows and swifts) and bats (31). Where many insects are emerging from a highly contaminated site, the possibility of contamination of these predators could be high. Whilst mosquitoes were used here as a model organism, any freshwater insect that can ingest MPs will likely equally transmit plastics into a terrestrial adult stage. This has implications for organisms that feed on adult mosquitoes with aerial and terrestrial animals accordingly open to MP exposure and transference would appear to occur at a higher rate for smaller MPs.

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Figure legends

Figure 1. Uptake counts of microplastics (MP) across larval (a, b), pupal (c, d) and adult (e, f) *Culex* mosquito stages following single (a, c, e) and mixed (b, d, f) exposures to 2µm and 15µm beads. Means are \pm SE ($n=5$ per experimental group).

Figure 2. Epi-fluorescent microscope images showing fluorescent microplastic particles within (A) the abdomen of an adult mosquito before dissection, and (B) the abdominal Malpighian tubules following dissection.

Ethics

Ethics committee approval was not required.

Data accessibility

Data files are available in online supplementary material.

Author contribution

All authors provided substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; were involved in drafting the article or revising it critically for important intellectual content; approved the final version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing interests

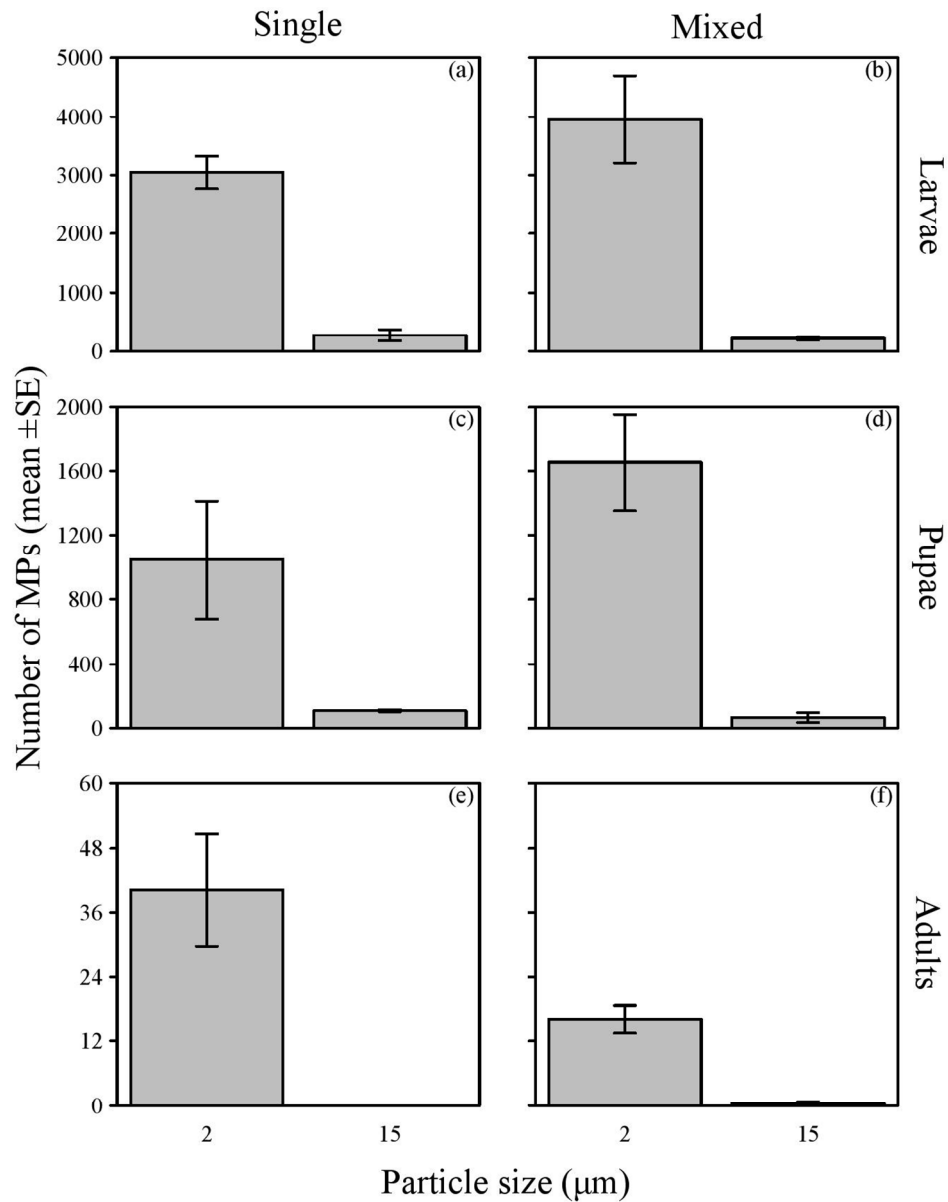
We declare we have no competing interests.

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